

Renal and retinal effects of enalapril and losartan in type 1 diabetes

Mauer *et al.*, *N Engl J Med* 2009; **361**:40–51

Since the initial demonstration that medications affecting the renin–angiotensin axis improved kidney disease related to diabetes,¹ trials have tested these medications earlier and earlier in the disease course.^{2–4} Similarly, the Daily-Dose Consensus Interferon and Ribavirin: Efficacy of Combined Therapy (DIRECT) trial⁵ reported that angiotensin receptor blockade reduced the rate of retinopathy development in normotensive patients with type 1 diabetes who did not have abnormal albumin excretion rates or retinopathy. A multicenter trial by Mauer *et al.* sought to confirm the benefit to retinopathy seen in the DIRECT trial and extend the findings of prior studies on type 1 diabetes and the progression of nephropathy. In separate arms, the authors examined the effect of enalapril and losartan, compared with placebo, on structural and functional changes within the kidneys and on development of retinopathy. Patients had type 1 diabetes mellitus and were normotensive, without abnormality in either creatinine or urinary albumin excretion. In 285 subjects, no difference was found between treatment arms for median albumin excretion rate, blood pressure, and glomerular filtration rate. Structural changes related to diabetes existed in the kidney biopsies of those enrolled at baseline before intervention. However, there was no difference in the progression or regression of these lesions across treatment arms. Surprisingly to the authors, enalapril did not change albumin excretion rate over 5 years as compared with placebo; however, losartan significantly increased mean albumin excretion rate as compared with placebo. Both enalapril and losartan significantly reduced the progression of retinopathy by both moderate and severe definitions.

The fact that the Renin–Angiotensin System Study (RASS) was similar to DIRECT in finding a benefit in retinopathy confirms that the lack of benefit within the kidney is not related to decreased power or other causes of a type II statistical error. While research continues to examine whether the use of these medications is warranted in the prevention of retinopathy, their use in the prevention of albuminuria and structural changes within the kidneys of type 1 diabetics is not.

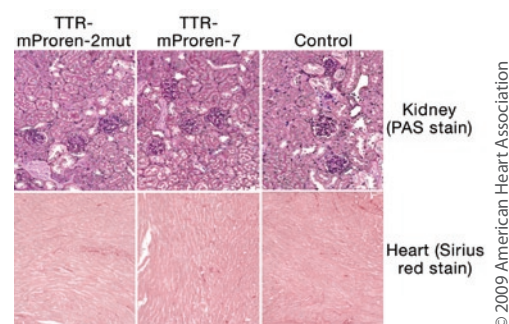
Lynda Szczech

¹*N Engl J Med* 1993; **329**: 1456–1462. ²*N Engl J Med* 2001; **345**: 861–869. ³*N Engl J Med* 2001; **345**: 851–860. ⁴*N Engl J Med* 2001; **345**: 870–878. ⁵*Lancet* 2008; **372**: 1394–1402.

Chronic increases in circulating prorenin

Mercure *et al.*, *Hypertension* 2009; **53**: 1062–1069; doi:10.1161/HYPERTENSIONAHA.108.115444

Renin is synthesized as a precursor, prorenin, a portion of which is converted to active renin by the proteolytic removal of an amino-terminal segment before its secretion by the renal juxtaglomerular cells. Prorenin is also present in the blood plasma at levels 5 to 10



Tissue effects of chronic hyper-proreninemia. In 18-month-old male control and transgenic mice, there was no increase in glomerulosclerosis (top) or cardiac fibrosis (bottom) in the animals with elevated levels of prorenin.

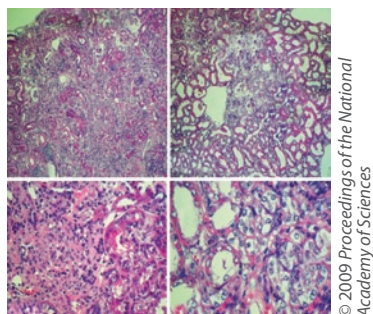
times those of renin and is produced not only by the kidney but also by other tissues. Prorenin has less than 1% of renin's enzymatic activity, but it appears to contribute to local angiotensin generation as it acquires enzymatic activity on binding to the (pro)renin receptor. In addition, both prorenin and renin can trigger intracellular mitogen-activated protein kinase activity by binding to this receptor. Prorenin levels increase with age; in diabetic patients, it has been postulated that elevated prorenin levels may cause disease. To test this, Mercure *et al.* generated transgenic mice with selective increases (13- to 66-fold) in circulating native or active site–mutated prorenin. They found that systolic blood pressure was either unchanged or increased (+25 mm Hg) in mice expressing native prorenin, whereas the mice expressing active site–mutated prorenin showed no change. The kidneys from the most highly prorenin-expressing lines revealed no evidence of injury but did show a slight increase in the urine content of albumin. Renal glomerular sclerosis did not increase in any of the transgenic animals tested, even in 18-month-old mice (Figure). In addition, although some evidence of cardiac hypertrophy was seen in the hypertensive animals, there was no increase in cardiac fibrosis. These results suggest that the primary consequence of chronic elevations in circulating prorenin is an increase in blood pressure, which does not support a role for prorenin as the primary causative agent in cardiac fibrosis or renal glomerular injury.

Juan Oliver

A mouse model of renal carcinoma

Morris and McClatchey, *Proc Natl Acad Sci USA* 2009; **106**: 9767–9772; doi:10.1073/pnas.0902031106

Renal-cell carcinomas (RCCs), which derive from renal tubule epithelial cells, are by far the most frequent kidney tumors. While most RCCs are sporadic, a few hereditary syndromes are associated with RCC. Somatic mutations in the genes associated with these syndromes have been identified in sporadic RCC, but a surprisingly low number of recurrent genetic alterations have been identified in RCC patient samples, and only three genes were found mutated at a frequency greater than 4%: *VHL*, 42%; *CDKN2A*, 12%; and *KIT*, 8%. That the homozygous mutation of the neurofibromatosis type 2 (*NF2*) tumor suppressor gene was identified in approximately 2% of



All *Vil-Cre;Nf2^{lox/lox}* mice (left panels) developed small intratubular neoplastic growths that grew to large tumors by 3 months and progressed to invasive carcinomas. This kidney phenotype was recapitulated in inducible *Mx1-Cre;Nf2^{lox/lox}* mice (right panels).

RCC patients was considered significant by Morris and McClatchey, especially in light of their previous work showing that the *Nf2* tumor suppressor Merlin is a critical regulator of epidermal growth factor receptor (EGFR). It has long been known that EGFR and its ligands, EGF and transforming growth factor- α , are frequently overexpressed in many forms of RCC. In a variety of conditions, the *Nf2* tumor suppressor Merlin coordinates the inhibition of EGFR signaling with the establishment of stable adherens junctions, thus mediating contact-dependent inhibition of proliferation. Morris and McClatchey thus hypothesized that *Nf2* mutation could generate renal tubular epithelial tumors. They first generated mice with a targeted deletion of *Nf2* in the proximal convoluted tubule using a *Villin-Cre* transgene and found that all these mice developed intratubular neoplasia that progressed to invasive carcinoma (Figure, left panels). To ascertain whether these renal tubule tumors derived from a developmentally and/or regionally specific cell population, they deleted *Nf2* in the collecting duct and the thick ascending loop of Henle of the adult kidney using the interferon- α -inducible *Mx1-Cre* transgene. These mice also developed lumen-filling renal tubule neoplasia (Figure, right panels). Interestingly, early lesions exhibited hyperactivation of EGFR signaling, altered solubility of adherens junctions components, and loss of epithelial polarity. Renal cortical epithelial cells derived from either early or late lesions formed malignant tumors upon subcutaneous injection into immune-compromised mice. Hence, targeted loss of the *Nf2* tumor suppressor in renal tubule epithelial cells gives rise to tumor development with 100% penetrance. These studies give further support to the role of EGFR signaling in RCC, suggesting that *Nf2* deficiency may be an important contributing factor in generation of these tumors and may provide a very useful model to study renal neoplasia.

Juan Oliver

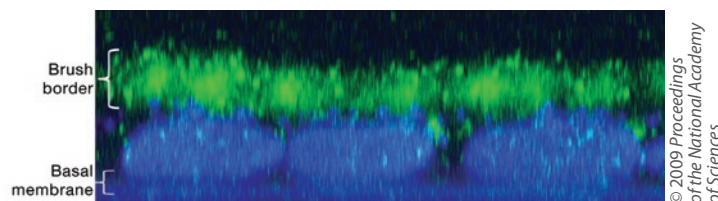
ABCG2 is a renal urate transporter with a common functional polymorphism causing gout

Woodward *et al.*, *Proc Natl Acad Sci USA* 2009; **106**: 10338–10342; doi:10.1073/pnas.0901249106

Genome-wide association studies (GWASs) have successfully identified common single-nucleotide polymorphisms (SNPs) associated

with a wide variety of complex diseases, including hypertension and chronic kidney disease. However, with rare exceptions, these studies do not address gene function or establish causality of the disease-associated SNP. Serum urate levels are highly heritable, and the main cause of elevated plasma urate levels is decreased renal urate excretion. Several renal urate transporters have been characterized, but their role in human disease is mostly unclear. Furthermore, despite much work and because of its complexity, renal urate transport is still poorly understood. In a GWAS of serum urate levels, multiple SNPs associated with urate levels and prevalence of gout were recently identified in a genomic region on chromosome 4 containing the ATP-binding cassette, subfamily G, member 2 (*ABCG2*) gene. *ABCG2* was initially identified as a multidrug-resistance protein and was found to transport a wide range of structurally and functionally diverse substrates. However, the physiological substrate and the role(s) of *ABCG2* *in vivo* have remained elusive. In a recent study, Woodward *et al.* expressed human *ABCG2* in *Xenopus* oocytes. They found that human ATP-binding cassette, subfamily G, 2 (*ABCG2*) is a hitherto unknown urate efflux transporter. Urate accumulation was markedly decreased in oocytes expressing *ABCG2*, and these oocytes had lower urate concentrations than oocytes expressing the known urate efflux transporter MRP4. In addition, the reduced urate accumulation in *ABCG2*-expressing oocytes was absent in the presence of a specific *ABCG2* inhibitor, or after introduction of a mutation in *ABCG2* that is known to disrupt its function. To analyze the urate-transport capacity of endogenous *ABCG2* in polarized renal epithelia, the authors measured urate accumulation in LLC-PK₁ cells. Inhibition of *ABCG2* caused a significant impairment of urate export from the cells and higher intracellular urate accumulation. In polarized LLC-PK₁ cells, *ABCG2* was localized to the apical brush border membrane (Figure), which is consistent with the recent demonstration of *ABCG2* in the brush border of human proximal tubule cells. To test whether the most significant SNP of the GWAS of serum urate levels (rs2231142 in exon 5 of *ABCG2*) was not only statistically associated but causally related to elevated urate levels, the authors introduced the mutation Q141K, encoded by the rs2231142 T allele, by site-directed mutagenesis. They found that Q141K-expressing oocytes had markedly reduced urate transport rates and decreased urate efflux across a range of intracellular urate concentrations. Hence, Q141K was shown to be a causal loss-of-function variant. Analysis of subjects from the GWAS further supported causality of rs2231142. These findings are of considerable clinical interest because of the high prevalence of the Q141K mutation in individuals of European and Asian ancestry. *ABCG2* therefore represents a potential drug target.

Juan Oliver



ABCG2 (green) detected in the apical brush border membrane of LLC-PK₁ cells. Nuclear DAPI stain (blue).